

CLAIMS

1. A method of detecting, and optionally selecting,  
5 a DNA sequence, characterized in that the DNA sequence to be detected possesses a stable expression-enhancing quality, which method comprises the steps of

1) the cloning in a vector of DNA fragments having a size of <5000 base pairs between i) a DNA sequence involved  
10 in the induction of gene-transcription repressing chromatin, and ii) a reporter gene comprising a promoter, resulting in a variety of a fragment-comprising vectors, wherein the distance between the DNA sequence involved in the induction of the transcription of  
15 gene-repressing chromatin and the reporter gene is fewer than 5000 base pairs.

2) introducing the vectors into host cells, in which host cells the promoter may be active but induction of the transcription of gene-repressing chromatin in the  
20 vectors results in the repression of the transcription of the reporter gene; and

3) subjecting the host cells to a selection in order to identify a host cell exhibiting reporter gene-activity.

25 2. A method according to claim 1, characterized in that the selection in step 3) occurs by using a reporter gene which provides resistance to a growth inhibitor and the host cells are cultivated in the presence of the growth inhibitor.

30 3. A method according to claim 2, characterized in that the growth inhibitor is present in a concentration sufficiently high to kill host cells in which the gene providing resistance to the growth inhibitor is not active.

35 4. A method according to claim 2 or 3, characterized in that an antibiotic is used as the growth inhibitor and the reporter gene is a gene providing resistance to the antibiotic.

5. A method according to claim 1, characterized in that the reporter gene codes for Green Fluorescent Protein.

6. A method according to claim 5, characterized in that fluorescent host cells are separated from non-fluorescent host cells by means of a Fluorescence-Activated Cell Sorter (FACS).

7. A method according to claim 1, characterized in that the reporter gene is luciferase.

8. A method according to ~~any of the preceding~~ claims, characterized in that the fragments have a size of substantially between 2000 - 3000 base pairs.

9. A method according to ~~any of the preceding~~ claims, characterized in that the DNA sequence involved with the transcription induction of gene-repressing chromatin is a DNA sequence that is recognized by a heterochromatin-binding protein comprising HP1 which HP1-comprising complex is expressed in the host cell.

10. A method according to ~~any of the claims 1 to 8,~~ characterized in that the DNA sequence involved with the transcription induction of gene-repressing chromatin is a DNA sequence that is recognized by a complex comprising a Polycomb-group (Pc-G) protein, and the Polycomb-group protein-comprising complex is expressed in the host cell.

11. A method according to ~~any of the claims 1 to 8,~~ characterized in that the DNA sequence involved with the transcription induction of gene-repressing chromatin is a DNA sequence that is recognized by a complex possessing a histone deacetylase activity, and the histone deacetylase activity-possessing complex is expressed in the host cell.

12. A method according to ~~any of the claims 1 to 8,~~ characterized in that the DNA sequence involved in the transcription induction of gene-repressing chromatin is a DNA sequence that is recognized by a protein complex comprising MeCP2 (methyl-CpG-binding protein 2), and the MeCP2-comprising complex is expressed in the host cell.

13. A method according to ~~any of the preceding~~ claims, characterized in that the DNA sequence involved with the transcription induction of gene-repressing

chromatin is a DNA sequence that is selectively recognized by at least one DNA-binding protein and the organism also expresses a protein complex comprising i) a first part selectively binding the DNA sequence, and ii) a second  
 5 part inducing the formation of chromatin in which the transcription is repressed.

14. A method according to claim 13, characterized in that the protein complex comprises a fusion protein.

15. A method according to claim 14, characterized  
 10 in that the first part is a part binding the DNA-binding site of LexA-DNA or GAL4-DNA.

16. A method according to any of the preceding claims, characterized in that the organism in step 1) is selected from the group comprising a plant and a verte-  
 15 brate.

17. A method according to claim 16, characterized in that the vertebrate is a mammal.

18. A method according to ~~any of the preceding~~  
 claims, <sup>8</sup> characterized in that the vector is an episomally  
 20 replicating vector.

19. A method according to ~~any of the preceding~~  
 claims, <sup>18</sup> characterized in that the vector comprises a  
 replication origin from the Epstein-Barr virus (EBV),  
 OriP, and a nuclear antigen (EBNA1).

20. A DNA sequence selected from i) a DNA sequence  
 25 isolated from a plant or vertebrate, or derivatives thereof, and ii) a synthetic DNA sequence or one constructed by means of genetic engineering, which DNA sequence is a repression-inhibiting sequence which, by the method accor-  
 30 ding to the present invention can be detected, selected and optionally cloned.

21. A DNA sequence selected from i) a DNA sequence isolated from a plant or vertebrate, or derivatives thereof, and ii) a synthetic DNA sequence or one constructed by  
 35 means of genetic engineering, which DNA sequence is detected, selected and optionally cloned by means of the method according to ~~any of the claims 1 to 19.~~  
<sup>18</sup>

22. A method of making a DNA construct comprising a gene that is to be expressed stably, wherein a stable

a expression-promoting DNA sequence is integrated in accordance with claim 20 or 21 in fewer than 2000 bp of the gene.

23. A method according to claim 22, characterized  
5 in that the stable expression-enhancing DNA sequence will be integrated both upstream and downstream from the gene.

a 24. A use of the DNA construct obtained in accordance with claim 22 or 23, wherein the DNA construct is a vector for the transformation of an organism.

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